

The Effects of Iodine Deficiency on Thyroid Hormone Deiodination

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Iodine deficiency induces multiple intrathyroidal autoregulatory changes leading to an increased triiodothyronine (T_3) production and secretion, at the expense of thyroxine (T_4). It is characterized by low serum T_4 , normal or slightly elevated T_3 , and as a consequence of the latter, normal thyrotropin (TSH). Tissues are also hypothyroxinemic, but their T_3 concentrations are mostly normal and ensure clinical euthyroidism, except for those that depend to a high degree on local generation from T_4 by extrathyroidal mechanisms involving the iodothyronine deiodinases isoenzymes. Thus, unless iodine deficiency is so severe and chronic that intrathyroidal and extrathyroidal mechanisms are no longer sufficient to maintain a normal T_3 in most tissues, individuals are clinically and biochemically euthyroid, but some tissues may be selectively hypothyroid (i.e., the brain). In adults both the intrathyroidal and the extrathyroidal mechanisms reacting to the iodine deficiency are fully operative even when the latter is mild. They contribute jointly to the maintenance of elevated or normal T_3 in those tissues deriving most of it from the plasma, until iodine deficiency becomes very severe. Those depending to a large extent from local generation from T_4 , mostly by an interplay between type 2 iodothyronine deiodinase (D2) and type 3 (D3), may already be T_3 -deficient (and hypothyroid) with mild iodine deficiency. Therefore, thyroid status of the iodine-deficient individual not only depends on the degree of iodine shortage, but is mostly tissue-specific, and is difficult to define for the individual as a whole: elevated, normal, and low concentrations of T_3 are found simultaneously in different tissues of the same animal, even with severe deficiencies. Most effects of iodine deficiency are reversed in the adults with an adequate iodine prophylaxis, but the absence of T_4 during early fetal life leads to irreversible brain damage (neurologic cretinism). Thyroid hormones of maternal origin are available to the embryo early in development and continue contributing to fetal thyroid hormone status, even after onset of fetal thyroid secretion. In the case of congenital hypothyroidism and normal maternal T_4 , the transfer of the latter, together with increased D2 activity, protects the fetal brain from T_3 deficiency, even when it may be insufficient to maintain euthyroidism in other fetal tissues. Practically all of the T_3 found in the fetal brain is derived locally from T_4 , and not from circulating T_3 . In the case of severe iodine deficiency, both the embryo and the mother are T_4 -deficient; therefore, the fetal brain is exposed to T_3 -deficiency, both before and after onset of fetal thyroid function. This leads to irreversible alterations and damage to the central nervous system (i.e. abnormal corticogenesis). Moreover, because intrathyroidal autoregulatory mechanisms are not yet operative in the fetus, both T_4 and T_3 continue to be very low until birth, and the fetus is not only hypothyroxinemic, similar to its mother, but also clinically and biochemically hypothyroid.

Introduction

IODINE IS AN ESSENTIAL micronutrient required for the synthesis of thyroid hormones. Iodine is very abundant in the sea but is very scarce in many areas of the world. Despite its scarcity and the fluctuations in its availability, throughout evolution vertebrates have not been able to free themselves from the need for iodine-containing hormones. Instead, they have developed specialized mechanisms to maintain a sta-

ble secretion of thyroid hormones when confronted with situations of iodine deprivation. During prolonged periods of severe iodine deficiency these mechanisms could become insufficient to meet the thyroid hormone requirements. Most of our understanding on the adaptation to iodine deficiency has been obtained using animal models (mainly rat, sheep, and marmoset, a primate) maintained on low iodine diets (LID) of variable degrees of deficiency (1–6). These models help us to understand the situation and health problems of

the people living in areas of endemic goiter, caused by iodine deficiency.

One of the main problems when summarizing our present knowledge about iodine deficiency is the diversity of animal models used, the different strains, variable length of the treatments, and the different composition of the diets that have been used. A brief comparison of the great experimental variables between different reported studies (1,7–28) highlights the many factors that influence the corresponding results and conclusions. The tracer amounts of other components of the diet (i.e., selenium [Se], vitamins) could also influence the response of the deiodinases in different organs (29–31) and therefore the metabolism of thyroid hormones. All these variables should be taken into account.

Despite all these differences in the severity of the iodine deficiency and in the presence of possible confounding factors, both in animals and in epidemiologic studies, it has become increasingly evident that thyroid autoregulation plays a prominent role in situation of inadequate iodine intake, and also that the extrathyroidal mechanisms may contribute to the maintenance of normal intracellular triiodothyronine (T_3) concentrations—and ensuing biologic actions—as long as possible.

We review the changes caused by iodine deficiency in thyroid hormone metabolism, especially through deiodinating pathways, in adults and during fetal and postnatal life, as well as the effects on early brain development, the irreversible neurologic manifestations, and the relevance of these findings for humans. As a major part of the studies related to iodine deficiency in different experimental animals have been performed before deiodinase activities and expression could be assessed directly, they are not discussed extensively here, and the reader is referred to previous reviews for the rat, sheep, and marmoset (1,3,32).

Most of the alterations in thyroid hormone metabolism described above could be reversed by the administration of iodide in the diet using the adequate amounts of iodine, except for the irreversible insults to the fetal brain presumably occurring at early stages of development. So it is evident that the study of the events occurring during gestation in the fetus and specifically in the developing brain deserve closer attention.

Iodine Deficiency in Adults

Thyroid autoregulation

Both in experimental animals and in humans the initial and immediate response to a decreased availability of iodine is the triggering of very efficient autoregulatory mechanisms, such as the increase in vascularity and in iodine uptake. Despite this, if the decreased availability of circulating iodide persists, there is a progressive increase in the ratios of iodothyrosines (monoiodotyrosine [MIT] to diiodotyrosine [DIT]) and iodothyronines (T_3 to thyroxine T_4), leading to the preferential synthesis of T_3 in the thyroid (11,14,20,33). There is a decrease in the proportion of mature fully iodinated (19 S) thyroglobulin (Tg) and an increase in poorly iodinated forms that leak more easily into the bloodstream (20). It appears that the intrathyroidal deiodination of T_4 into T_3 further increases the preferential secretion of T_3 . Thus, increased serum T_3/T_4 ratios and Tg are typical markers of these autoregulatory mechanisms. An unexpected finding

was that all these changes are independent of thyrotropin (TSH; 34–35), occurring when hypophysectomized animals are fed a low iodine diet, whether or not they are substituted with TSH. The increase in thyroid weight and volume, that are part of the autoregulatory changes, do not necessarily mean that circulating TSH is, or has been, increased. The rapid autoregulatory response to a decreased iodine intake has been confirmed in humans (36).

As a result of the autoregulatory mechanisms only circulating T_4 decreases, but serum T_3 does not, and might even increase (37–38), thus preventing the increase in serum TSH and the clinical signs of hypothyroidism. Elevated TSH is rarely found in goitrous individuals from areas with iodine deficiency alone (38,39).

Hypothyroidism versus hypothyroxinemia

Iodine deficiency in humans has been frequently and inaccurately associated with hypothyroidism and increased TSH (40), whereas many individuals from iodine-deficient areas are clinically euthyroid (41) and their TSH is not elevated because their circulating T_3 is normal, or even slightly elevated, as the immediate consequence of the thyroid's autoregulatory mechanisms (38–39,42). These misconceptions are likely to be the result of different causes. The important role played by TSH-independent autoregulation is often overlooked, or actually not known to younger Western-trained physicians. Moreover, no clear distinction was made between the iodine deficiency disorders (IDD) reported in areas of iodine deficiency alone and those reported from regions where other additional factors, such as goitrogens and/or selenium deficiency, result in atrophy of the thyroid and overt clinical and biochemical hypothyroidism (43,44). With uncomplicated iodine deficiency, overt signs and symptoms of clinical or subclinical hypothyroidism (increased serum TSH) are not observed, because many tissues derive their intracellular T_3 from their normal or slightly elevated circulating T_3 . But it does not mean that these euthyroid individuals might not suffer from selective hypothyroidism of organs, such as the brain, that derive an important part of the intracellular T_3 by local deiodination from T_4 (40,45–46). The iodine-deficient fetuses and newborns are an important exception, because thyroid autoregulation is not yet fully operative (47) and they are clinically and subclinically hypothyroid, as is described in more detail later.

The condition presenting with a decrease in plasma T_4 , without an increase in circulating TSH, has been termed hypothyroxinemia (46), and for this reason we shall refer to iodine-deficient individuals and animals as hypothyroxinemic, not as hypothyroid. This assumes that their tissues would be hypothyroxinemic, but that the tissue concentrations of T_3 would be normal, or even elevated. However, this is not necessarily so, as extrathyroidal adaptive mechanisms involving the different responses of the iodothyronine deiodinases (D1, D2, and D3) to changes in the availability of T_4 and T_3 , exert a further important role (1,3,32).

Role of the iodothyronine deiodinase isoenzymes

The mechanisms of maintenance of T_3 include changes in the deiodinases (D1, D2, and D3), which respond to the hypothyroxinemia present in tissues of the adult iodine-defi-

cient rat (23,30–31). As expected D2 activities increase in cerebral cortex (24,25,31,48), cerebellum, brown adipose tissue (23,24,30) and pituitary (30), while D1 decreases in liver, kidney, lung, and pituitary and increases in the thyroid (24; unpublished results). The decrease in D1 activity in liver is not observed with milder iodine deficiency (23,25).

An important point regarding the maintenance of plasma T_3 within normal levels is whether, or not, mechanisms are involved, other than the preferential synthesis of T_3 by autoregulation. This was investigated (23) comparing the thyroidal and hepatic D1 and brown adipose tissue D2 in rats on LID. D1 was increased in the thyroid, which seems to be the main source of serum T_3 , as the liver D1 activities hardly changed, and it is unlikely that the increased brown adipose tissue activities contribute to the maintenance of normal serum levels in LID rats (23).

Moreover, the deiodinases are selenoenzymes and therefore a nutritional deficiency of selenium will influence the thyroid status in iodine deficiency. Selenium deficiency leads to decreases of liver D1 and D3 (31), more pronounced than those induced by iodine deficiency. The combined deficiency of selenium and iodine does not lower the increased D2 activity in brain (31), possibly because of the different selenium content in liver and brain.

Severe iodine deficiency

In severely iodine-deficient rats, the brain is T_3 -deficient and biologic end points of thyroid hormone action are affected, such as the density and distribution of dendritic spines of the pyramidal neurons from the visual cerebral cortex (21), in agreement with the decrease of T_3 in the nuclear fraction. This was to be expected, considering the importance of T_4 for local generation of T_3 in the brain. Less expected was the finding that in severely iodine-deficient adult rats, other tissues considered as mostly dependent on plasma-derived T_3 for their intracellular T_3 , were also T_3 -deficient (49) and functionally hypothyroid, despite circulating T_3 that was still normal. Several end points of thyroid hormone action were, for instance, affected in the liver, such as α -glycerophosphate dehydrogenase and malic enzyme activities (20) in accordance with the decreased T_3 in the nuclear fraction. The growth hormone (GH) content of the pituitary was also decreased (20). In general, it might be concluded that in severely iodine-deficient adult rats, a normal circulating T_3 does not necessarily mean that all tissues that are mostly dependent on serum-derived T_3 are actually euthyroid.

Heterogeneity of cerebral structures

Of great interest are the distributions of D2 and D3 in the different areas of the brain, that are not homogeneous. The expression of D2 is more intense in the hypothalamus (tanyocytes and median eminence) and pituitary (50–54), with transient increases having also been described in the mouse cochlea at postnatal day 7 (55). The most extensive up-to-date study regarding the expression and activities of D2 and D3 in different brain areas of the iodine-deficient adult rat is that of Peters et al. (56). Using *in situ* hybridization techniques they have shown that iodine deficiency increases D2 mRNA and D2 activity, especially in cerebral cortex, medial basal hypothalamus, cerebellum, hippocampus, and pituitary, whereas D3 mRNA and activity decrease, especially in

cerebral cortex, hippocampus, and cerebellum. Whereas reductions of D3 expression and activity in different regions of the iodine-deficient rat brain were of a similar magnitude, a great discrepancy was systematically observed between the increases in D2 expression and D2 activities. The latter increases greatly exceeded those expected from the changes in D2 mRNA, drawing attention to the important contribution of posttranslational effects of the low intracellular T_4 of the iodine-deficient animal. The mechanism by which T_4 , the preferred substrate for D2, reduces its protein levels is the consequence of the T_4 -induced increase in the rate of D2 ubiquitination and subsequent proteosomal degradation (57). Thus, when T_4 falls, as is the case in iodine deficiency, the D2 half-life is prolonged and the D2 activities exceed those expected from the increase in D2 mRNA. All the changes described (56) would result in a higher production of T_3 from T_4 (through increased D2 activity), enhanced by the reduced degradation of T_3 and T_4 (through decreased D3), all tending to mitigate in the brain the effects of the hypothyroxinemia caused by iodine deficiency by maintaining normal intracellular T_3 concentrations. However, how successfully this is achieved in different cerebral regions was not assessed. It will depend on the respective availability of the substrate, T_4 , and therefore ultimately on the degree of iodine-deficiency.

Iodine Deficiency Early in Development

Iodine deficiency is a worldwide public health problem and the most important cause of preventable mental retardation. Approximately 1000 million people are living in conditions of chronic iodine deficiency of varying degree (58) and the World Health Organization (1990) has estimated that 20 million people are suffering from preventable brain damage.

There are several situations in which alterations of the central nervous system (CNS) have been related to impairment of the thyroid function during fetal development (for reviews see Morreale de Escobar et al. [32],46]. Among the different conditions the most severe damage in the CNS is found in neurologic cretinism, associated with severe iodine deficiency during the first trimester of pregnancy. The damage can only be avoided by supplying adequate amounts of iodine to the mother, before or early in pregnancy. Neurologic cretinism is a more severe condition than congenital hypothyroidism and is irreversible by birth, at which time the administration of iodine, or thyroid hormone, is no longer fully effective.

The severe neurologic manifestations in endemic cretinism point to CNS damage that has occurred early during fetal life. The most frequent alterations are deafness; motor deficits such as spasticity, trunk rigidity, flexion dystonia and muscle wasting; and mental deficiency (intellectual and visuomotor integration deficits, autism, vacuity) (59,60). Some of them, such as the loss of hearing, point to damage in the cochlear development occurring during the first trimester of pregnancy. Mild and moderate degrees of iodine deficiency are also adverse for the outcome of the pregnancy (61). For years it was difficult to understand the role of thyroid hormones during early periods in which the fetal thyroid function is still not functional, but today much experimental evidence points to the role of the maternal thyroid

hormones in early brain development during fetal life. Over the last 20 years animal experiments carried out in several laboratories have obtained evidence for a role of the maternal thyroid hormones, and very especially of T_4 , during early fetal development.

Thyroid hormones and deiodinases before onset of fetal thyroid hormone secretion

The evidence derived from the rat model may be summarized as follows.

T_4 and T_3 are found in embryonic and fetal tissues (62,63) before the onset of the fetal thyroid secretion that in the rat starts at 17.5-18 days of gestation. The earliest day tested is 9 days of gestation, which is 4 days after implantation, and these concentrations remain constant until the onset of fetal thyroid secretion. Functional nuclear T_3 receptors are also detected in the fetal rat brain by 14 days of gestation (64), by *in situ* hybridization the $TR\alpha_1$ isoform is present in the fetal brain at 14 days of gestation (65) and the $TR\beta_1$ at 11.5 days of gestation in the neural tube. The β_1 and β_2 transcripts are already detected at 12.5 days of gestation in an area that will be very important for the auditory system (66,67). The expression of the TR isoforms suggests important roles of thyroid hormones in specific areas during brain development.

The T_4 and T_3 found in the early embryos are of maternal origin, because their concentrations are very low in the case of maternal hypothyroidism (63,68) and in the fetuses from mothers fed an LID (69). Figure 1 compares the T_4 and T_3 concentrations in embryotrophoblasts, placentas, and embryos found in fetuses from mothers on LIDs before the onset of fetal thyroid function. T_4 and T_3 are very low in embryotrophoblasts, embryos and placentas obtained from LID mothers compared to their respective controls (similar to the

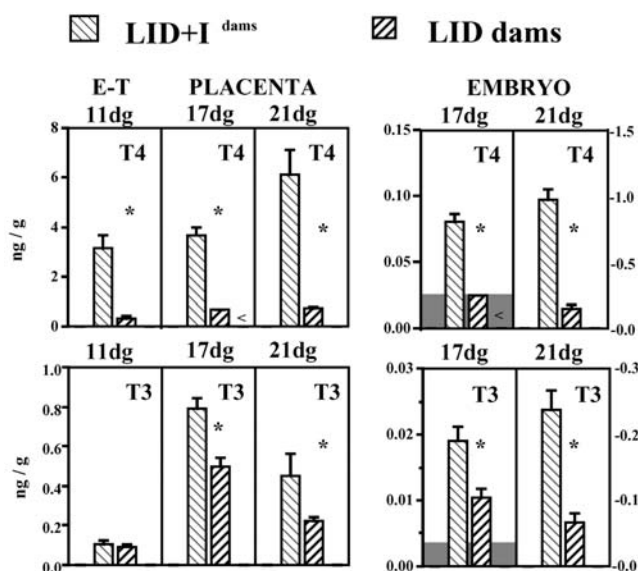


FIG. 1. Thyroxine (T_4) and triiodothyronine (T_3) concentrations (means \pm standard error of the mean [SEM]) in embryotrophoblasts (E-T), placentas and embryos from dams fed iodine-deficient (LID) and iodine-supplemented (LID+I) diets. The dotted areas at the bottom of each panel indicate the limits of detection of the T_4 and T_3 assays. Data are from Escobar del Rey et al. (69).

T3 in fetal brain and whole fetus **Effect of increasing doses of T_4 and T_3**

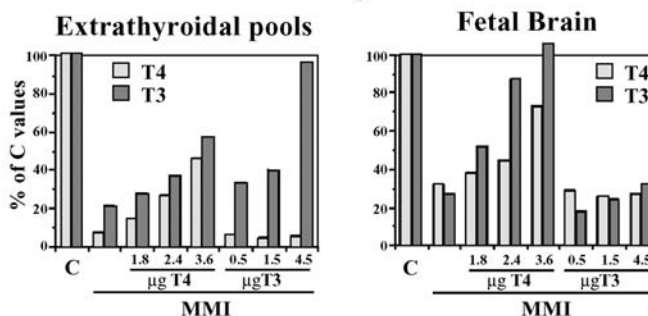


FIG. 2. Changes in the concentrations of thyroxine (T_4) and triiodothyronine (T_3) in 21-day-old fetuses from normal (C) and 2-mercapto-1-methylimidazole (MMI)-treated dams, infused with placebo or increasing amounts of T_4 or T_3 (doses are $\mu\text{g}/100$ g of body weight [BW] per day). The extrathyroidal pools comprise the amounts of T_4 and T_3 in the whole fetus, after dissecting out the thyroid. Data are given as percentage of values in normal (C) fetuses. The daily dose of $2.4 \mu\text{g } T_4$ per 100 g BW was adequate to maintain maternal euthyroidism. It was not sufficient to ensure normal T_4 and T_3 pools of the fetus as a whole, or T_4 of the fetal brain, but was enough to ensure normal brain T_3 in the brain of the MMI-fetuses. The infusion of T_3 at a daily dose of $0.5 \mu\text{g}$ per 100 g BW maintained maternal euthyroidism but did not ameliorate fetal brain T_3 deficiency. Data are from Calvo et al. (72).

findings in embryonic structures from thyroidectomized (T_x) mothers).

Thyroid hormones and deiodinases after onset of fetal thyroid hormone secretion

Maternal transfer in normal and hypothyroid conditions. The maternal transfer of thyroid hormones continues until birth. At term the contribution of the mother represents approximately 20% of the total extrathyroidal pool of fetal T_4 in the rat (70), and up to 50 % of serum T_4 in the human fetus (40,71). The amount of T_4 transferred, although not enough to prevent thyroid hormone deficiency in all the tissues of the rat fetus at term, is enough to avoid the cerebral T_3 deficiency until birth in the case of fetal hypothyroidism (72). This was demonstrated in experiments in which hypothyroid rat dams on 2-mercapto-1-methylimidazole (MMI) were infused with increasing doses of T_4 or T_3 . The main findings are illustrated in Figure 2, which compares the T_4 and T_3 concentrations reached in extrathyroidal pools and in the fetal brain: A dose of $2.4 \mu\text{g } T_4$ infused to the mother normalizes T_3 in the fetal brain, even when T_4 in fetal brain is only 50% that of control. This is an experimental situation of interest for human congenital hypothyroidism, where the maternal contribution of T_4 would avoid the cerebral T_3 deficiency of the fetuses, protecting their brain until birth. As may be seen for the rat in Figure 2, cerebral T_3 remains low, despite an increase in circulating levels in the fetuses from the dams infused with high T_3 doses. These findings confirm that the fetal rat brain derives all its T_3 by local generation from T_4 , and none from circulating T_3 .

In the fetus the protection of the brain by T_4 is achieved through the increase in the type II deiodinase (D2) activity in response to the low circulating and cerebral levels of T_4 (72,73). The increased D2 activity produces more or less T_3 depending mostly on the amount of available substrate (T_4), whether the latter is of fetal or maternal origin. Therefore, during early fetal development an adequate supply of maternal T_4 is of primary importance, whichever the circulating levels of T_3 .

Fetal thyroid status in normal conditions. There is a large increase in the thyroidal T_4 and T_3 pools from 18 days of gestation until birth (100- and 400-fold, respectively) (74). As a consequence of this and of the continuing maternal transfer there is a 9-fold increase in the circulating T_4 , as well in most tissues, organs, and in the total extrathyroidal pool. In contrast, the circulating T_3 levels do not increase. T_3 concentrations, however, do increase in many fetal tissues and in the extrathyroidal pool and do so at different rates (74), except for the liver T_3 , which increases only 2- to 3-fold.

The changes in plasma and brain are shown in (Fig. 3, left). The concentrations of T_4 and T_3 in fetal brain increase very

quickly during the last 4 days before birth (73,74) (Fig. 3, left). Brain T_4 increases 9-fold in parallel with plasma T_4 , though at a quicker rate suggesting an increased uptake of T_4 by the fetal brain. The increase of T_3 (18-fold) is the result of the ontogenic increases in D2 activities that locally provide the T_3 required for the fetal brain. This is in contrast to the low increases in liver T_3 and D1 (not shown).

The amount of T_4 available to the fetus, whether of maternal or fetal origin, plays a limiting role for the local generation of T_3 in the fetal brain. Cerebral D2 activity is able to respond to hypothyroidism (MMI) as early as day 17 of gestation (73), that is, before the onset of fetal thyroid secretion. The increases in D2 activities are small at the earlier times tested (2-fold at 17 days' gestation) but increase later on (at 19–22 days' gestation). The presence of D2 in fetal brains earlier in gestation (10–16 days gestation) remains to be identified, it might be located in specific brain nuclei only visible by *in situ* hybridization techniques. D2 activities in rat fetal brain and its responses to hypothyroidism are similar to those found in adult brain, suggesting that the deiodinative mechanisms are fully operative.

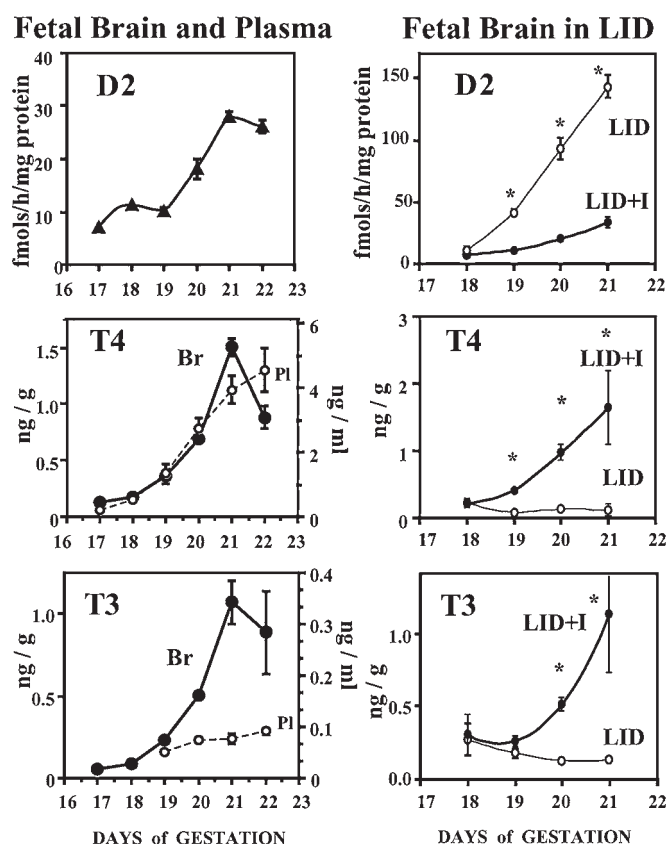


FIG. 3. Left: Type 2 iodothyronine deiodinase (D2) activities and concentrations of thyroxine (T_4) and triiodothyronine (T_3) in the brain of rat fetuses between onset of thyroid function (17 dg) and birth (22 dg). The T_4 and T_3 concentrations in fetal plasma are also depicted. Data are from Ruiz de Oña et al. (73). **Right:** Concentrations of T_4 and T_3 and D2 activities in the brain of rat fetuses (18–21 dg) from mothers on an iodine-deficient (LID) and iodine-supplemented (LID + I) diets. Data are from Obregon et al. (24). Data are mean \pm SEM standard error of the mean (SEM).

Fetal thyroid status in iodine deficiency. The impact of iodine deficiency for the fetal and early neonatal brain is illustrated in Figure 3, right, and Figure 4, in which thyroid hormone concentrations and D2 activities in fetal and neonatal brain of fetuses and pups on LID are represented at the end of gestation and during the postnatal period comprising the first 4 weeks of life (24). During the fetal period (Fig. 3, right) there is a large increase in D2 activity in response to iodine deficiency. It is not, however, able to increase T_3 in the fetal brain because of the very low concentration of T_4 , which is its only source of cerebral T_3 . The situation is different during the postnatal period (Fig. 4). The little iodine that is available to the dam is concentrated in the milk, because the mammary gland competes efficiently with the maternal thyroid to concentrate iodine, thus providing an increased amount of iodine to the lactating pups (24). This leads to a small increase in the circulating and brain T_4 (to 25%–35% of LID + I pups) that, together with the dramatic increase in D2 activity, produces T_3 in amounts similar to those found in the brain of iodine-sufficient pups (LID + I). The pattern of changes of brain D2, T_4 , and T_3 shows two clear peaks, one at the end of the fetal period and a second one 2 weeks after birth, which are coincident with important periods of brain development, occurring both in the LID and LID + I pups.

Other fetal tissues also showed signs of clear hypothyroidism. D1 activities were reduced in liver and lung of LID fetuses, even when the activities were very low compared to those of adult rats, while BAT D2 increased sevenfold in LID fetuses at term (24), up to levels found in cold-exposed rats. The fetal skin is another source of T_3 with increased D2 activities during iodine deficiency (48). D3 activities decreased in fetal skin of LID fetuses at the end of gestation (48). Until now, no changes in the placental D2 and D3 have been reported in response to iodine deficiency. The fetal thyroid responds to mild and severe iodine deficiency with increases in the expression of sodium iodide symporter (NIS) and D1 mRNA (75). The expression of NIS was also found in the fetal side of the placenta and was increased in LID fetuses (75), suggesting the participation of the pla-

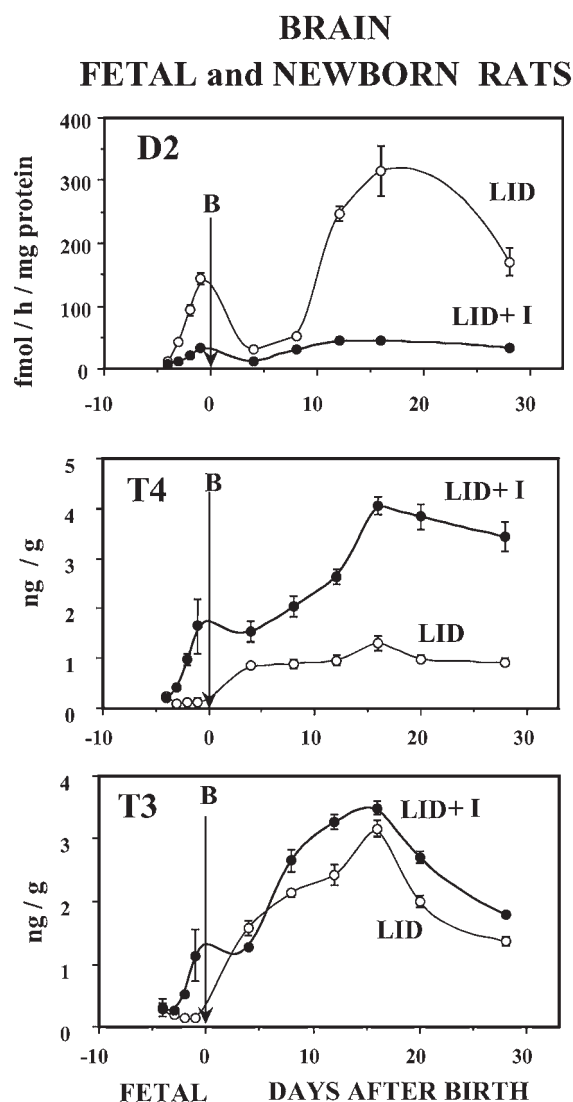


FIG. 4. Concentrations of thyroxine (T_4) and triiodothyronine (T_3), and type 2 iodothyronine deiodinase (D2) activities in the brain of rat fetuses and pups until 4 weeks of postnatal age born from mothers on an iodine-deficient (LID) and iodine-supplemented (LID + I) diet. The data shown "before" birth are the same of Figure 3. Data (mean \pm standard error of the mean [SEM]) are from Obregon et al. (24).

centa in the iodine-concentrating mechanisms functioning during gestation.

In summary, the consequences of iodine deficiency during gestation are as follow: the transfer of maternal thyroid hormones to the fetus is very low during the early period of gestation, because the mother is hypothyroxinemic. During the final period of gestation the maternal transfer continues to be very low and the fetus is unable to produce enough T_4 for the normal brain development, because of the shortage of iodine. After birth iodine is concentrated in the milk and increases the iodine available to the suckling pup's thyroid, as well as the circulating and brain T_4 that, combined with the increased D2 activity, is able to mitigate the T_3 deficiency observed in the neonatal brain.

Irreversible neurologic abnormalities

Evidence from experimental animals. Several neurologic abnormalities have been demonstrated in rats born from mothers fed markedly iodine-deficient LID diets for one or two generations (76,77). The behavioral defect found is the susceptibility to audiogenic seizures, associated with hearing defects typical of neurologic cretinism. We have recently confirmed this in the progeny of mothers on LID, and the defect is irreversible, even when the iodine deficiency is corrected during adult life, strongly suggesting that an early deficiency in maternal T_4 , leading to decreased fetal brain T_3 , induces irreversible alterations in cerebral development.

Many morphologic end points of development of the cerebral cortex, hippocampus, and cerebellum have been reported in the progeny from dams chronically on LID (i.e., see Martinez-Galan et al. [78] for the rat and Hetzel [3] for the sheep and marmoset). Recently we have shown that maternal hypothyroxinemia restricted to early stages of development affects neurogenesis irreversibly. Two models have been studied: the iodine-deficient rat dams (LID model) (79) and dams (3d-MMI model) that were treated for only 3 days with a goitrogen (2-MMI), which resulted in a transient and moderate maternal thyroid hormone deficiency (80). In both models the dams were hypothyroxinemic without being hypothyroid during the period of active corticogenesis and migration of radial neurons into the developing cerebral cortex and hippocampus. This developmental phase occurs when the mother is the only source of thyroid hormone available to the fetus. The final location of those cells generated during early periods of neurogenesis was determined in the young rats. The cells were labeled with bromodeoxyuridine (BrdU), injected into the dams during gestation (between embryonic day 14–16 or embryonic day 17–19), when two major waves of radial migration of neurons into the somatosensory cortex are taking place. With both models the migration of the BrdU-immunoreactive cells, most of which were neurons, was affected, with neurons appearing in aberrant locations, such as the subcortical white matter, and in cortical and hippocampal layers inappropriate for their date of birth. The proportion of neurons reaching the outer layers of the somatosensory cortex was clearly decreased. Cytoarchitectural changes were also evidenced in the hippocampus and barrel cortex of the primary somatosensory cortex. Maintenance of maternal hypothyroxinemia throughout pregnancy (LID model) was not necessary for the appearance of these abnormalities, as only a short period of maternal thyroid hormone deficiency between embryonic day 12–15 (3d-MMI model) was sufficient to derange the radial migration of neurons generated between embryonic day 14–16 and those generated between embryonic 17–19, as well as the cytoarchitecture of the hippocampus and barrel cortex. In the 3d-MMI model the maternal thyroid hormone deficiency was quite moderate and limited to a very precise window during development. Correction of the moderate maternal thyroid hormone insufficiency by infusion of T_4 during MMI treatment prevented the alterations of radial neuronal migrations and cytoarchitecture described above, whereas T_4 infusion was of no benefit when delayed beyond this critical period of corticogenesis. An unexpected finding was the induction of behavioural alterations in the pups born to the treated dams

of the 3d-MMI model: an increased susceptibility to respond with wild runs to acoustic stimulation.

Such experimental findings clearly support that an adequate supply of maternal thyroid hormone is very important for the early neurodevelopment of the fetus. If relevant to humans, they might help to define the period in human gestation when the fetal cerebral cortex is especially sensitive to changes in the availability of maternal thyroid hormone. In humans, the radial migrations of neurons into the cortex take place mostly between the 8th and 24th weeks postconception, with two main waves peaking at the 9th and 12th weeks, namely at 11 and 14 weeks postmenstrual age (PMA). The first one coincides approximately with the peak of the maternal human chorionic gonadotropin (hCG)-driven serum free fT_4 surge.

Evidence from studies in humans. Most of the evidence indicating that early maternal hypothyroxinemia caused by iodine deficiency affects early brain development in human has been obtained from abundant epidemiologic associations obtained from population studies, and has been considered indirect evidence. Very recently, however, an important observation has been published that constitutes direct proof, because the evidence has been obtained by pairing data on the neurodevelopment of the child with maternal thyroid status early in pregnancy. Vermiglio et al. (81) have found a statistically significant correlation between the IQ of the child at 9–10 years of age and the maternal thyroxinemia during the first (but not later) trimester of pregnancy. Both the early maternal hypothyroxinemia and the decrease in IQ were relatively moderate, as was the degree of iodine deficiency, but 70% of the children from the early hypothyroxinemic mothers and 0% of nonhypothyroxinemic mothers were diagnosed as having attention deficit hyperactivity disorder (ADHD).

The human fetal brain

Although for many years the human fetus has been considered to develop in the absence of thyroid hormones, there is increasing evidence that most of the findings summarized here for rat in experimental models might be applied to man.

We now have evidence that in the human fetuses T_4 is found in fluids from the embryonic cavities (coelomic and amniotic cavities) during the first trimester of gestation. T_4 in coelomic fluid, and later in fetal plasma, is correlated positively with maternal plasma T_4 (82,83). Both T_4 and T_3 and the nuclear TRs are found in the human fetal brain by the end of the first trimester of gestation and increase 10-fold by 18 weeks' gestation (84,85). The expression of $TR\alpha$ and $TR\beta$ isoforms has been recently reported in the cerebral cortex of first trimester fetuses (8–14 weeks) (86,87).

T_4 and T_3 are found in cerebral cortex, liver and lung of human fetuses of 8–18 weeks' PMA (84,85). The ontogenic patterns of T_4 , T_3 , rT_3 , D2 and D3 activities in nine different cerebral areas from fetuses of 13,20 weeks PMA have recently been reported (88). They showed spatial- and temporal-specificity with divergence between the cerebral cortex and other cerebral areas, such as the cerebellum, as illustrated in Figure 5. The concentrations of T_4 , T_3 , and the activity of D2, increase with age in the cortex between 13–20 weeks PMA, whereas rT_3 (not shown) decreases. D3 activity is very low.

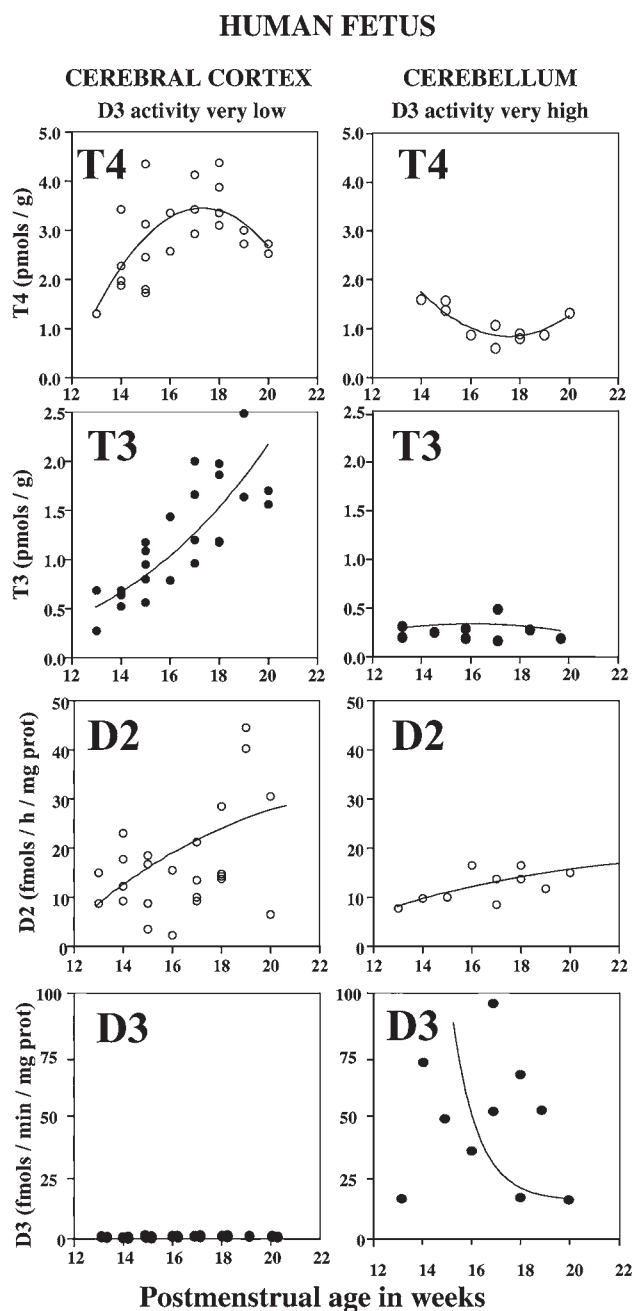


FIG. 5. Ontogenic changes in the concentrations of thyroxine (T_4), triiodothyronine (T_3), type 2 and 3 iodothyronine deiodinases (D2 and D3) activities in the human fetal cerebral cortex and cerebellum from 13th weeks up to midgestation. During this period T_4 in the fetal serum increases approximately fivefold and circulating T_3 remains very low. Data are from Kester et al. (88).

In the cerebellum D3 activities are very high, T_3 concentrations are low, and T_4 does not increase. Concentrations of rT_3 (not shown) are higher than in cerebral cortex. Other regions also have high D3 activities (brain stem, spinal cord, midbrain) and low T_3 concentrations. These findings support the need of T_3 by the human cerebral cortex before midgestation, when the mother is the sole source of precursor T_4 and confirm the importance of D2 and D3 in determining

the local availability of T_3 at the appropriate timing. Others have also reported the expression of D2 in fetal cerebral cortex as early as 7–8 weeks PMA with a peak at 15–16 weeks (89). Results so far confirm the important roles of D2 and D3 in the local bioavailability of cerebral T_3 during fetal life: D2 generates T_3 from T_4 and D3 protects brain regions from excessive T_3 until differentiation is required. This is in conceptual agreement with a recent report in *Xenopus laevis* showing that D2 is present at the precise time that a given tissue responds to T_3 -induced metamorphosis (90), being the deiodination of T_4 a precise requirement in this process.

To our knowledge there is only one report from Karmarkar et al. (1993) that analyzes the effect of iodine deficiency in human fetal brain. They measured T_4 , T_3 , rT_3 , D2, and D3 in the cerebral cortex of human fetuses (11–25 weeks' gestation) from women with mild to moderate iodine-deficiency mothers and in fetuses from iodine-sufficient mothers (91). T_4 and T_3 concentrations peaked at 15–18 weeks' gestation and then decreased in fetuses from iodine-sufficient groups, while in mild iodine deficiency T_3 concentrations were maintained at a higher level until week 22, although they were always lower than in the iodine-sufficient group. rT_3 rose from 11 to 22 weeks' gestation with no effect of iodine status. D2 and D3 activities increased from 11 to 22 weeks' gestation, and D2 increased further in mild and moderate iodine deficiency, whereas D3 activity was significantly decreased. It appears interesting that D2 activity had not yet increased in response to the lower T_4 at 11–14 weeks' gestational age, suggesting that downregulation of D2 activity by T_4 is delayed with respect to the onset of its expression. It did so later, at 15–18 weeks, but was, however, inadequate because T_3 in the cortex remained low in iodine-deficient conditions. Therefore, cerebral cortex D2 and D3 activities are modulated in iodine deficiency to enhance T_3 production from T_4 during the period of active corticogenesis in the human brain.

There is increasing evidence of the expression of the deiodinases in the utero-placental unit and D3 in the fetal epithelia (92,93), but their possible alterations in iodine-deficient conditions are unknown.

Adaptation to Different Degrees of Iodine Deficiency

We have already pointed out in the introduction that it is very difficult to compare results from the many studies carried out in rats fed diets with "a low iodine content," especially because its actual iodine content is usually not reported, and because of the many other confounding factors, other than iodine intake, that may affect the findings. From the survey of results from animal experiments and epidemiologic reports in humans, it may be concluded that the actual degree of the iodine deficiency is crucial for the final results, with both intrathyroidal and extrathyroidal mechanisms contributing differently according to the availability of this micronutrient.

We have recently attempted to define the sequence of intrathyroidal and extrathyroidal adaptations to different degrees of iodine deficiency in young adult female rats fed for 3 months with the same Remington-type LID diet. On the basis of an intake of 20 g/d, this diet provided 0.06 μg I. Different groups received the same diet, supplemented with added potassium iodide to provide a further 0.5, 1.0, and 5.0 μg of iodine per day. Another group, LID, was fed LID to which minute amounts of KClO_4 (0.005%) were added to inhibit the availability to the thyroid of the small amounts of iodine contained in the diet. This amount was likely to be reduced from 0.06 $\mu\text{g}/\text{d}$ to approximately 0.03 $\mu\text{g}/\text{d}$. Thus, there was a more than 100-fold range of iodine intakes between the LID + 5.0 and LID groups. The total weight and iodine content, T_4 and T_3 contents and D1 activities were measured in the thyroid, as well as plasma T_4 , T_3 , and TSH, and T_4 and T_3 concentrations in 11 different tissues, and D1 or D2 activities in 4 of them. Some of the findings are illustrated in Figures 6 and 7.

Intrathyroidal autoregulatory mechanisms are already clearly effective when the iodine intake and thyroid iodine content were reduced from the LID + 5.0 to the LID + 1.0 groups, including a preferential secretion of T_3 at the expense of T_4 , and an increase in thyroid weight. All these changes occur without a statistically significant increase in circulat-

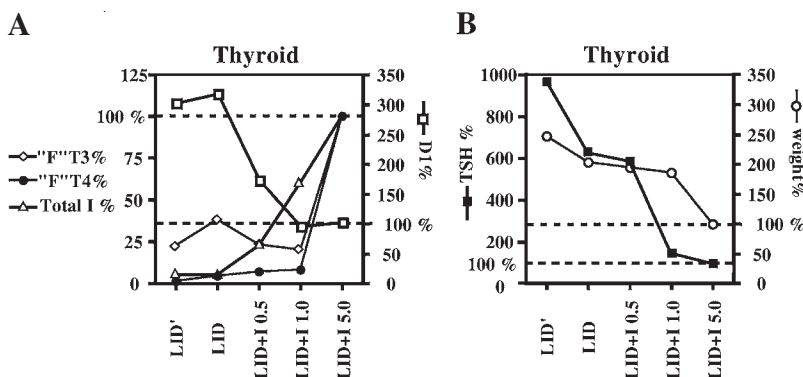


FIG. 6. Changes in the thyroid gland of adult female rats fed for 3 months with the same low-iodine diet (LID) and different iodine supplements, from 0.03 (LID' group) to 5 μg of iodine per day (LID + 5.0 group). (A) shows the mean total iodine, "Free" thyroxine (T_4) and "Free" triiodothyronine (T_3) contents, and D1 activities of the thyroid gland, expressed as percentage of the mean value found for the LID + 5.0 group. The "Free" T_4 and "Free" T_3 contents correspond to the concentrations of T_3 and T_4 present as iodoaminoacids (not incorporated into proteins by peptidic bonds) and available for secretion. B: Thyroid weight is significantly increased without an increase in circulating thyrotropin (TSH), as well as the intrathyroid "Free" T_3 to "Free" T_4 ratio, without the increase in D1 observed with a more marked degree of iodine deficiency (A).

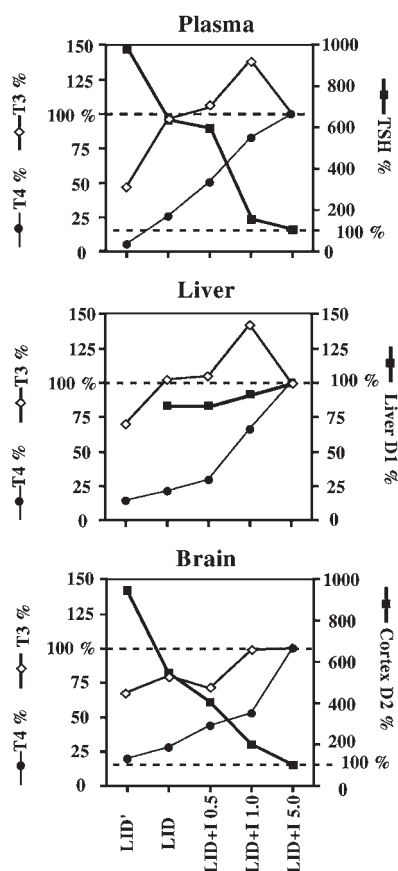


FIG. 7. Changes observed in the plasma, liver, and brain of the same rats as those of Figure 6. Consistent with the findings shown in the latter for the thyroid, the autoregulatory mechanisms triggered with the decrease in iodine intake from the LID + 5.0 to LID + 1.0 groups, there is a rapid increase of the preferential secretion of triiodothyronine (T_3) into the plasma, at the expense of thyroxine (T_4), without an increase in thyrotropin (TSH), that starts increasing in those groups with a lower iodine intake, where circulating T_3 is no longer elevated. The changes in the concentrations of T_4 and T_3 in the liver were consistent with those observed in the plasma, with a minor decrease of D1 in the LID + 1.0 and LID groups. Despite the very marked increase in D2 activity in the brain, with increasing hypothyroxinemia, cerebral T_3 started decreasing with iodine intakes between those of the LID + 1.0 and LID + 0.5 groups, although it is likely that this occurs at different degrees of iodine deficiency for different cerebral structures (56).

ing TSH. One of the extrathyroidal adaptative mechanisms, namely the increase in cerebral D2, was also triggered by this minor degree of iodine deficiency and cerebral T_3 was maintained within the normal range. An increase in thyroid D1 activity does not appear to be involved in the initial autoregulatory responses, as the increase already described by others (23) was only found when iodine intake was further decreased (LID + 0.5 group) and plasma TSH had increased significantly. In this group, cerebral T_3 concentrations were decreased, despite the further increase in D2, whereas liver T_3 was still normal, and only decreased with the most extreme iodine deficiency (LID group). Although not illustrated here, some tissues (i.e., ovary, lung, and muscle) were

able to maintain normal concentrations of T_3 , even in the LID group, where plasma T_3 was reduced to 50% of normal values. The results of this study stress that the thyroid autoregulatory response to iodine deficiency predominates as an adaptative mechanism when the individual is faced with moderate chronic decreases of iodine intake. The extrathyroidal response of cerebral D2 activity contributes to the maintenance of adequate concentrations of T_3 in the brain. In most tissues T_3 concentrations may actually increase. With a further decrease in iodine availability the thyroid autoregulatory mechanisms no longer suffice and extrathyroidal adaptation involving the iodothyronine deiodinases plays an increasingly important role, but may also become insufficient for some, but not all, tissues. The thyroid status of the iodine-deficient rat is not only dependent on the degree of iodine availability but is also tissue-specific, and this could account for the differences in reported results, even for the same species. Thus, a single answer cannot be given to the question of whether iodine-deficient individuals are euthyroid or hypothyroid, even if their plasma T_4 , T_3 , and TSH are known, because of the involvement of metabolic pathways—mainly deiodinative—that also appear to be tissue-specific. Even with the most severe iodine deficiency that we have studied, tissues with decreased, normal or increased T_3 concentrations coexist in the same animal. This contrasts with the situation encountered in cases of primary thyroid failure (thyroidectomy, treatment with goitrogens, etc.) where autoregulatory mechanisms are impaired. Thus, the involvement of the deiodinating isoenzymes in thyroid hormone metabolism and thyroid hormone status is not necessarily the same as that described in primary hypothyroidism, and has to be studied in the iodine-deficient animal in a tissue-specific manner, at different levels of iodine intake and, as we have already seen, at different stages of development.

Summary

From the present studies we conclude that iodine deficiency leads to important thyroidal and extrathyroidal adaptations aimed to maintain T_3 concentrations in tissues. Many intrathyroidal autoregulatory changes occur even without an increase of circulating TSH and result in a preferential synthesis and secretion of T_3 . Maintenance of normal or elevated T_3 at the expense of T_4 maintains clinical euthyroidism, although some tissues (i.e., brain) might be selectively T_3 -deficient and hypothyroid. In contrast to the condition of the euthyroid iodine deficient mother, these autoregulatory adaptative mechanisms are not yet fully developed in the fetus. Its circulating T_3 is very low after onset of fetal thyroid secretion, as a result of which the fetuses and newborns from iodine-deficient mothers are T_3 -deficient throughout gestation and clinically hypothyroid. This is why in areas of iodine deficiency TSH values at neonatal screening are higher than those of newborns from iodine-sufficient areas, and their frequency may be used as criterion for the identification of regions with varying degrees of this micronutrient deficiency.

With increasing severity of the iodine deficiency, the degree of the hypothyroxinemia also increases and autoregulatory mechanisms become insufficient for maintenance of normal T_3 in the circulation and most tissues. Extrathyroidal responses involving the iodothyronine deiodinases are trig-

gered progressively and play a crucial role in determining the thyroid hormone status in a tissue-specific fashion. There is a very important increase in the activity of D1 in the thyroid, that enhances the secretion of T_3 generated from T_4 , and that contributes to maintenance of normal plasma T_3 . Especially important for the thyroid hormone status of specific tissues, such as brain and BAT, is the interplay between the increased activity of D2 in response to the low T_4 , and the concomitant decrease in that of D3, sparing T_4 and prolonging the half-life of intracellular T_3 . The final condition is not only dependent on the degree of iodine deficiency but is tissue specific. Even in rats on a very low iodine intake, that is 100-fold lower than that required for normal thyroid function, tissues with elevated, normal, and decreased T_3 are found in the same animal, with biologic end points of thyroid hormone action affected accordingly.

It is quite unlikely that human populations would survive in a certain geographical area when thyroid autoregulation and other adaptive mechanisms would no longer suffice to maintain T_3 within normal limits. The iodine intake in areas of severe iodine deficiency is still approximately one tenth of requirements, contrasting with the 100-fold decrease that may be obtained in experimental animals. In these human populations women are severely hypothyroxinemic, but maintain euthyroidism at the expense of normal plasma T_3 levels, despite which fetal neurodevelopment may be more or less severely compromised. When unable to do so and both circulating T_4 and T_3 are low, fetal loss is the outcome, and populations would eventually abandon the area, or disappear. Thus, findings from severely iodine-deficient animals should not be extrapolated to the human situation without taking into account the degree of hypothyroxinemia and whether, or not, circulating T_3 is still normal.

Iodine deficiency, and especially that of the pregnant woman, is still at the beginning of the third millenium, the major cause of mental retardation worldwide, affecting millions to a greater or lesser degree. Despite the many sustained efforts of many international institutions, such as UNICEF, World Health Organization, and International Council for the Control of Iodine Deficiency Disorders (ICCIDD), this human scourge still exists, despite the ease with which iodine-deficiency disorders can be avoided by providing sufficient iodine to the whole population, and extra iodine supplements during pregnancy and lactation. Europe is far behind other continents in these efforts and there is recent evidence that an increasing proportion of North American pregnant women do not receive the optimal amounts of iodine and require supplementation.

Remember that despite all the intrathyroid and extrathyroidal mechanisms in which iodothyronine deiodinases play a very important role, they may be insufficient to protect the brain in iodine deficiency, and the utmost efforts are needed to comply worldwide—including industrialized societies—with the following basic human rights:

1. Every child has the right to an adequate supply of iodine to ensure his (or her) normal development. Of particular importance in this context is the right of the unborn child.
2. Every mother has the right to an adequate iodine nutrition to ensure her unborn child experiences normal mental development.

(Declarations emanating from the Convention on the Rights of the Child, United Nations Assembly, New York, 1989; World Summit for Children, United Nations, New York, 1990.)

Acknowledgments

This is written in memory of John T. Dunn, who dedicated all his life to worldwide prevention of iodine-deficiency disorders.

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